Sleep Medicine 15 (2014) 892–898

Contents lists available at ScienceDirect

Sleep Medicine

journal homepage: www.elsevier.com/locate/sleep

Original Article

An fMRI study of cerebrovascular reactivity and perfusion in obstructive sleep apnea patients before and after CPAP treatment

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ARTICLE INFO

Article history:
Received 19 December 2013
Received in revised form 5 April 2014
Accepted 10 April 2014
Available online 4 May 2014

Keywords:
Obstructive sleep apnea
OSAS
CPAP
Functional magnetic resonance imaging
fMRI
Hypoxemia
Cerebral vascular reactivity
Cerebral perfusion

ABSTRACT

Objective: Cerebrovascular reactivity is impaired in patients suffering from obstructive sleep apnea syndrome (OSAS) as demonstrated by transcranial Doppler studies. We use magnetic resonance imaging techniques to investigate the anatomical distribution of cerebrovascular reactivity changes in patients with OSAS, as well as their evolution after therapeutic and sham continuous positive airway pressure (CPAP) treatment.

Methods: Twenty-three men with moderate or severe obstructive sleep apnea were compared to a healthy control group ($n = 7$) using a breath-holding functional magnetic resonance imaging task and the flow-sensitive alternating inversion recovery (FAIR) imaging before and after 2 months of therapeutic (active) or sub-therapeutic (sham) CPAP treatment.

Results: Significantly higher cerebrovascular reactivity was found in healthy controls as compared to patients in bilateral cortical and subcortical brain regions. Cerebrovascular reactivity increased with therapeutic CPAP in the thalamus and decreased with sham CPAP in medial frontal regions in OSAS patients. Duration of nocturnal hypoxemia and body mass index negatively correlated with cerebrovascular reactivity, particularly in the medial temporal lobe structures, suggesting a possible pathophysiological mechanism for hippocampal injury.

There was no difference in perfusion between patients and control group, and no effect of CPAP or sham-CPAP treatment on perfusion in patients.

Conclusions: Observed cerebrovascular reactivity changes were neither homogeneous throughout the brain nor followed vascular territories, but rather corresponded to underlying neuronal networks, establishing a relationship between cerebrovascular reactivity and surrounding neuronal activity.

1. Introduction

Obstructive sleep apnea syndrome (OSAS) is a breathing disorder occurring during sleep in at least 2–4% of the adult population, and is associated with increased risk of neurocognitive impairment, sleepiness, and mood and cardiovascular disorders [1–3]. Whereas OSAS was found to be an independent risk factor for stroke, hypertension, and cardiac arrhythmias, the mechanisms responsible for those effects remain incompletely understood. Previous research established that OSAS leads to impairment of cerebrovascular regulation and deficiency of endothelial function, resulting in decreased cerebrovascular reactivity (CVR) to chemical (hypercapnia, hypoxia) and mechanical stimuli [4–6]. Furthermore, the degree of CVR impairment was linked to the degree of severity of OSAS-related nocturnal hypoxemia [7]. CVR is considered an index of capability of cerebral vessels to adapt to the metabolic demand of the brain and to maintain adequate perfusion in the presence of fluctuation in oxygen and carbon dioxide blood levels. In association with other conditions predisposing to stroke (such as cardiac arrhythmias and metabolic syndrome), CVR impairment along with blood pressure and rheological changes appear to be the major OSAS-related factors for the genesis of cerebrovascular comorbidities.

Previous research of CVR changes in OSAS patients was primarily based on inferences from breath-holding (BH)-associated changes in cerebral blood velocity measured by trans-cranial Doppler ultrasound [8,9], but the advent of magnetic resonance neuroimaging techniques enabled a whole-brain approach to the study of CVR changes. In the recent past, BH functional magnetic

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http://dx.doi.org/10.1016/j.sleep.2014.04.004
1389-9457/

resonance imaging task (BH-fMRI) was successfully used to investigate CVR and regional variability of cerebral blood oxygenation response to hypercapnia in healthy young adults, during normal aging and in children [10–16]. BH-fMRI uses the increase of arterial PCO_2 induced by a BH maneuver as a vasodilative stimulus in the absence of cognitive function's variation in order to assess CVR. It was shown that BH fMRI-induced signal change correlates well with results of CVR modifications assessed by single photon emission computed tomography study with acetazolamide challenge [17], an important observation when investigating CVR in pathological conditions.

In contrast to middle cerebral artery (MCA) examinations by trans-cranial Doppler, the whole-brain fMRI approach allows investigation of anatomical distribution of CVR differences in the brain and across all vascular territories. This is of particular interest since there is increasing body of evidence suggesting that OSAS is associated with regionally specific structural and metabolic brain changes. These changes were most consistently observed in the hippocampus, seemingly related to hypoxic damage and sleep fragmentation, two consequences of OSAS [18–23].

Investigation of regional CVR with fMRI may add a new dimension to our understanding of the pathophysiologic mechanisms underlying the hypoxemic-related functional and structural changes observed in the brain of OSAS patients.

In this study, we use two different techniques, the BH-fMRI and the flow-sensitive alternating inversion recovery (FAIR) imaging, to evaluate the anatomical distribution of CVR differences and cerebral perfusion in OSAS patients in comparison to findings noted in healthy controls. Magnetic resonance imaging water spin-labeling techniques such as FAIR use water protons as endogenous tracers to measure cerebral blood flow and provide a non-invasive assessment of baseline cerebral perfusion unavailable with BH-fMRI-derived analyses of vascular reserve [24,25]. The measurement of basal perfusion provides an important variable: a resting baseline index of whole brain perfusion, against which regional CVR susceptibility can be referenced. Evaluation of both BH-fMRI and of FAIR offers us the means to investigate two distinct but complementary aspects of the vascular regulation: (a) CVR as measured by BH-fMRI reflecting vascular capacity for vasodilation to CO_2 stimulus, and (b) using FAIR technique, the cerebral blood flow (CBF) reflecting the baseline cerebral perfusion at rest, in the absence of evoked response or challenge.

The aims of our study are (a) to investigate regional CVR and baseline cerebral perfusion in untreated OSAS patients as compared to healthy controls, (b) to investigate regional CVR susceptibility to the duration of nocturnal hypoxemia, and (c) to uncover CVR changes after 2 months of therapeutic nasal continuous positive airway pressure (CPAP) as compared to sham-CPAP treatment.

We hypothesize that (a) OSAS patients will demonstrate compromised vascular reserve as reflected by decreased CVR, (b) that the severity of CVR decrease will correlate with the severity of OSAS-related nocturnal hypoxemia, particularly in medial temporal regions, sensitive to effects of hypoxemia, and (c) that CVR will be improved (increased) by therapeutic CPAP treatment.

2. Methods

2.1. Participants

Twenty-three men diagnosed with moderate to severe OSAS were recruited from the Stanford Sleep Clinic and surrounding area via advertisement. None of the patients had been previously treated with CPAP. All participants were right-handed nonsmokers and were screened for hypertension, cardiac and pulmonary disease, anemia, usage of vasoactive medication, brain trauma, as well as current or previous neurological and psychiatric disorder as determined by history, clinical evaluation, and Hamilton Depression Scale score. All participants reported regular sleep schedules with $\geqslant$6 h of sleep per night as determined by sleep habits questionnaires. Seven age-matched subjects without history of sleep disorders were recruited from the community as healthy controls; and absence of sleep pathology, including sleep disordered breathing, was confirmed by an overnight polysomnography (apnea hypopnea index (AHI) <5) (Table 1). This study was approved by the Stanford Institutional Review Board, and all subjects provided written informed consent prior to study enrollment.

Patients who were confirmed to have OSAS on diagnostic polysomnography were randomly assigned to either the active (therapeutic) or sham (sub-therapeutic) nasal CPAP group (n = 11 and n = 12, respectively). A CPAP titration study was conducted for patients in both groups, during which active group subjects were effectively titrated and sham group subjects slept with the sub-therapeutic nasal CPAP. The sham-CPAP device closely simulated the airflow through the exhalation port and the operating noise of the active-CPAP device. Prior study using a functionally similar sham-CPAP device revealed that oxygen saturation, end-tidal CO_2, and mean temperature and humidity measured at the CPAP mask were the same with sham-CPAP and no significant difference was found in sleep parameters or the number of abnormal respiratory events between sham-CPAP and no-treatment groups [26]. Subjects in both groups were treated for 2 months and treatment compliance was monitored using Encore® Pro Smart Card® system. The Encore® Pro Smart Card® system essentially works by providing a recording of CPAP device usage over a predetermined period of time (Table 1). At the end of the treatment period, sham-CPAP group subjects underwent a second CPAP titration night and left the study with assignment of the appropriate therapeutic CPAP treatment.

2.2. Polysomnography

Overnight polysomnographies were performed in all subjects. The following variables were systematically monitored: electroencepahlogram, electro-oculogram, electrocardiogram, chin and leg myogram, nasal airflow with nasal cannula, abdominal and thoracic respiratory movements with inductive plethysmography belts, and pulse oximetry. Studies were scored by independent technicians and reviewed by a qualified sleep medicine physician according to the American Academy of Sleep Medicine scoring criteria.

Table 1
Baseline patient groups' characteristics.

	Active-CPAP (n = 11)	Sham-CPAP (n = 12)	p Value
Age	43 (±7.7)	43.6 (±7.4)	0.9
BMI	28.9 (±4.5)	26.1 (±2.3)	0.07
AHI	49.6 (±21.9)	36.4 (±17.2)	0.12
ESS	8.2 (±5.8)	9.7 (±4.3)	0.5
TST	382.3 (±81.1)	376 (±44)	0.8
Sleep efficiency	83.1 (±15)	83.7 (±7.9)	0.9
REM	18 (±5)	19.8 (±4.2)	0.4
Stage 1	12.4 (±8.6)	11.3 (±6.7)	0.7
Stage 2	62.5 (±9.6)	61.7 (±9.5)	0.9
Stage 3+4	7.2 (±6.8)	7.2 (±7.5)	1
Minutes <90% SpO_2	64.7 (±120)	7.3 (±8.7)	0.2
Nights of CPAP usage	66.3 (±22.8)	62.3 (±23.1)	0.7
% of nights with >4 h CPAP usage	62.6 (±28.2)	68.5 (±31.8)	0.6

BMI = body mass index, AHI = apnea hypopnea index, ESS = Epworth Sleepiness Scale, TST = total sleep time, REM = rapid eye movement sleep.

2.3. Experimental procedures

All subjects were instructed to abstain from ingestion of any caffeinated beverages $\geqslant$9 h prior to scanning.

2.3.1. Breath holding–functional magnetic resonance imaging task (BH-fMRI)

Previous study by Thomason et al. demonstrated that BH-fMRI derived blood oxygen level-dependent (BOLD) signal variability can be significantly reduced with use of visually cued task in which subjects were given inspiration depth feedback [27]. We have used the same protocol with visual bio-feedback in order to reduce task variability due to inspirational volume differences and monitor the correct task performance in the scanner. All subjects were scanned in the evening.

Subjects wore a respiratory monitoring belt placed snugly around their upper thorax. The belt's electrical conductance is nominally proportional to the belt circumference and thus varied as the subject breathed. The belt was energized by a 5-V power supply through a 24-kΩ resistor and connected to a custom-made analog to digital (A/D) converter, which in turn was connected to the stimulus presentation computer as a parallel device. During the 3-s inspiration phase of the task, the A/D was strobed at 10 Hz and the reading at the end of the 3-s period was taken as the inspiration level for that trial.

All subjects were trained to correctly perform the task outside of the scanner. During the BH-fMRI scan, subjects' breathing pattern was continuously monitored by experimenters on the monitoring computer screen to insure correct task performance.

Subjects performed eight repetitions of alternating periods of BH after inspiration and self-paced breathing. Task blocks were cued by differently colored squares presented sequentially on a black screen. A green square was presented for 13.5 s, signifying normal breathing. The square turned yellow for 3 s, during which time the subjects were to take in a comfortable inspiration in preparation to hold. Then the square turned red for 13.5 s, signifying the BH maintenance period. The total cycle time was thus 30 s, and an additional 16-s regular breathing block was appended to make the total scan time 256 s exclusive of 6 s of dummy equilibrium frames at the beginning of the BH-fMRI scan. BH-fMRI task stimuli were presented using E-Prime (http://www.pstnet.com) software.

2.4. Magnetic resonance imaging acquisition parameters

Magnetic resonance imaging was performed on a 3.0-Tesla whole body scanner (Signa, rev 12M5, GE Healthcare Systems, Milwaukee, WI, USA) using a custom quadrature birdcage head coil. Head movement was minimized with foam padding and clamps attached to the coil. Thirty contiguous axial slices were obtained with 4-mm slice thickness. High-resolution T2-weighted fast spin echo structural images (TR = 3000 ms, TE = 68 ms, ETL = 12, FOV = 22 cm, matrix 192 $\times$ 256) were acquired for anatomical reference. A T2*-sensitive gradient echo spiral in/out pulse sequence [28,29] was used for BH fMRI (TR = 2000 ms, TE = 30 ms, flip angle = 77°, matrix 64 $\times$ 64, same slice prescription as the anatomic scan). A high-order shimming procedure was used to reduce B0 heterogeneity prior to the functional scans [30]. Spiral in/out methods have been shown to increase signal-to-noise ratio and (BOLD) contrast to noise ratio in uniform brain regions as well as to reduce signal loss in regions compromised by susceptibility-induced field gradients generated near air–tissue interfaces such as prefrontal cortex [28]. Compared to traditional spiral imaging techniques, spiral in/out methods result in less signal dropout and greater task-related activation in prefrontal cortex regions [31]. A high-resolution T1 volume scan (124 slices, 1.2 mm thickness) was collected for every subject using an IR-prep 3D FSPGR sequence for T1 contrast (TR 8.9 ms, TE 1.8 ms, TI 300 ms, flip angle 15°, FOV 24 cm, slice thickness 1.2 mm, matrix 256 $\times$ 192 $\times$ 128 mm). fMRI data were preprocessed using Statistical Parametric Mapping (SPM8) (Wellcome Department of Cognitive Neurology, London, UK) and custom MATLAB routines (MathWorks, Natick, MA, USA).

Cerebral perfusion was measured within the same magnetic resonance scan session. The FAIR sequence is obtained by using water protons as endogenous tracers of flow-sensitive movement and is described in detail elsewhere [24,25]. Participants were instructed to lie still with their eyes closed while they were scanned using FAIR for 4 min. Constraints of the FAIR method and optimal slice thickness (5 mm) required acquisition of a partial brain volume. One acquisition consisted of 10 axial slices, 5 mm thick (no gap), TR = 3 s, TE = 8 ms, TI = 1200 ms, flip angle = 90°, in-plane resolution 3.43 $\times$ 3.43 mm^2, and 80 time frames. The readout utilized a spiral k-space trajectory instead of the usual EPI method, allowing a short TE and short readout duration that minimized geometric distortion and signal loss from T2 decay. We used precisely the same imaging protocol described in previous work by Thomason and colleagues, utilizing the same magnetic resonance system and hardware [32].

2.4.1. Magnetic Resonance preprocessing and statistical analyses

BH-fMRI preprocessing steps consisted of realignment of all images to the first image, time slicing correction, co-registration to the individual high resolution anatomical image, normalization to Montreal Neurological Institute template, and spatial smoothing with a Gaussian filter of 8 mm full-width-half-maximum. Additionally, a voxel-level linear model of the global signal (LMGS) technique developed by Macey and colleagues was used to remove global effects from fMRI time-series [33] prior to statistical modeling.

Statistical analysis at the single-subject level treated each voxel according to SPM's general linear model. Regressors for the corresponding condition blocks (breath and hold) were modeled as a boxcar function convolved with the canonical haemodynamic response function. To this model, the six motion parameters from the realignment were added as six regressors of no interest.

For group level analysis, individual contrast images corresponding to "breath versus hold" were used (1) in a two-sample *t*-test to compare untreated OSAS and healthy control groups, (2) in a flexible factorial design in order to examine the group (sham vs. therapeutic CPAP) x scan (baseline vs. post-treatment) interaction, and (3) in post-hoc paired *t*-tests to compare baseline to post-treatment scans for each OSAS patient group (sham and therapeutic CPAP).

All BH-fMRI analyses were confined to a custom gray matter mask derived from high-resolution anatomical images of our subjects (size: 230,218 voxels) with between-condition statistical threshold set to $|t| > 2.47$(OSAS vs. healthy controls); $|t| > 2.58$ (multiple regression) (voxel level $p < 0.01$, uncorrected), cluster size >236 voxels, which corresponded to whole-brain voxel-level $p = 0.05$ corrected for multiple comparisons. Multiple comparison correction levels were determined by Monte-Carlo simulations, which were performed using the Alpha-Sim routine available in the REST software package [34,35].

Additionally, percent signal change was computed for regions of interest (ROIs, 5 mm spheres) centered on maximal intensity voxels of HC versus OSAS, pre- versus post-CPAP and pre- versus post-sham CPAP treatment analysis results (Fig. 2) using RfxPlot software [36].

To test for correlation of BH signal changes (CVR) with clinical measures, a multiple regression analysis was performed with AHI, time spent under 90% SpO_2 (minutes) during sleep, and body mass index (BMI) as covariates. These analyses were performed on the data from the OSAS patients only. Twenty out of 23 OSAS patients were included in multiple regression analysis as three subjects had incomplete overnight desaturation data.

3. Cerebral perfusion analysis (FAIR)

For FAIR perfusion analyses, for each subject, cerebral blood flow (CBF) maps were automatically generated by the scanner as part of that imaging sequence. Individual CBF maps were coregistered to individual anatomical images and normalized. Global mean CBF values were calculated for each subject and entered in the "global calculation" step in the group analysis. In the group level analysis, paired *t*-tests were used to compare OSAS patients to healthy controls and a flexible factorial analysis to assess treatment group versus scan session interaction (active and sham-CPAP effects) in OSAS patients.

In order to compare results from our imaging methods (BH-fMRI and FAIR) and to examine the relationship between changes in CVR and regional CBF (rCBF) values, individual gray matter ROIs were created from high-resolution anatomical images of each subject, corresponding to the acquired FAIR volume. Data series were extracted from those ROIs from rCBF and BH-fMRI images and submitted to correlation analysis.

All results are reported at the statistical threshold of $p < 0.05$ corrected for multiple comparisons (multiple comparison correction levels were determined by Monte-Carlo simulations, which were performed using the Alpha-Sim routine available in the REST software package [34,35]).

4. Results

4.1. CVR in patients versus healthy controls

CVR is measured as percent blood oxygenation level signal change (between "breath" and "hold" conditions of the BH-fMRI task). All reported results have reached the significance level of $p < 0.05$, corrected for multiple comparisons.

Significantly higher CVR was observed for the BH-fMRI task in healthy controls than in OSAS patients in the left lentiform nucleus, extending into the left pulvinar and parahippocampal gyrus, in the left post and precentral gyri and in bilateral superior frontal gyri (BA 8) extending into the left medial frontal gyrus (Table 2A, Fig. 1).

No brain regions had significantly higher CVR in OSAS patients as compared to healthy controls.

Table 2
Localization of CVR changes. Brain regions showing significantly bigger BOLD signal changes during breath-holding task in (A) HC as compared to OSAS patients; (B); brain regions showing significant correlation between CVR and AHI, nocturnal hypoxemia duration and BMI; (C) brain regions with significantly higher CVR after CPAP treatment as compared to baseline and significantly lower CVR after sham-CPAP as compared to baseline ($p < 0.05$, corrected for multiples comparisons, MNI coordinates).

BH	Region	Brodmann	*x*	*y*	*z*	*Z* score	Cluster size
A. HC>OSA	R sup/medial frontal	BA 8,6	8	24	56	3.78	538
	L lentiform nucleus/putamen/parahippocampal		−26	−14	−8	3.62	404
	L postcentral	BA 2	−44	−26	32	2.89	238
OSA>HC	None						
B. AHI positive	L PCC/precuneus	BA 31/30	−6	−64	12	3.38	346
AHI negative	R thalamus		18	−18	14	3.06	447
Desaturation positive	L precuneus/paracentral lobule	BA 5	−4	−38	64	3.04	340
Desaturation negative	R middle/inferior temporal/parahippocampal	BA 21,20	42	−20	−26	3.53	841
	R middle/sup frontal	BA 8.6	30	16	46	3.59	481
	R inf frontal	BA 47	38	32	−16	3.14	270
	L parahippocampal/hippocampus	BA 36	−34	−24	−24	3.4	240
BMI positive	None						
BMI negative	R parahippocampal		22	−42	−18	3.85	753
	L parahippocampal		−18	−44	−12	3.88	409
	L/R cingulate	BA 31,24	−4	−24	38	3.61	239
C. CPAP							
Post-treatment >baseline sham-CPAP	L/R thalami		−6	−10	2	3.44	281
baseline>post-treatment	L med frontal/ACC	BA 10,32	−6	56	−8	3.66	258

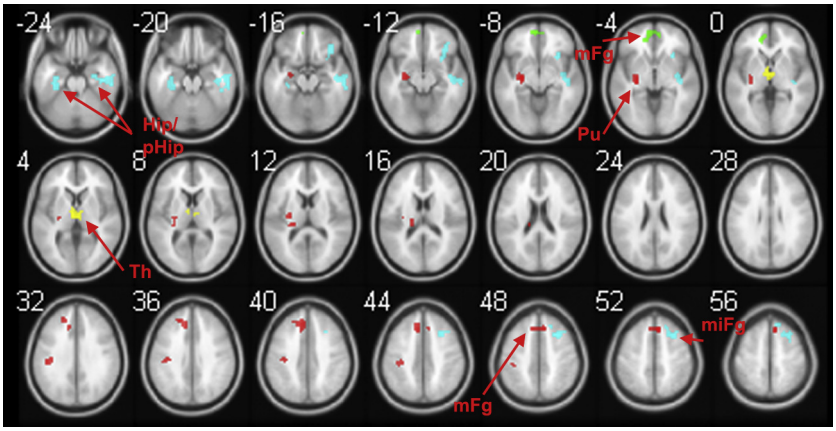

Fig. 1. CVR as reflected by changes in BOLD signal during the breath-holding task: (a) brain regions with higher CVR in HC as compared to OSAS patients (red); (b) brain regions showing significantly higher CVR after therapeutic CPAP treatment as compared to baseline (yellow); (c) brain regions showing significantly lower CVR after sham-CPAP treatment as compared to baseline (green); (d) brain regions showing significant negative correlation with the duration of nocturnal hypoxemia (blue) ($p < 0.05$, corrected for multiple comparisons, neurological presentation. Hip/PHip: hippocampus, parahippocampal gyrus; Th: thalamus; Ln: lenticular nucleus; medFg: medial frontal gyrus; mFg: middle frontal gyrus). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

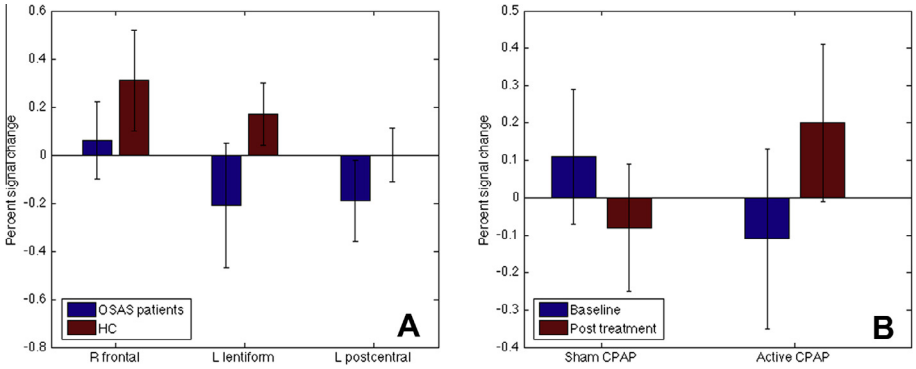

Fig. 2. Percent BOLD signal change within (a) anterior cingulate cortex ROI derived from the results of paired *t*-test analysis of sham CPAP group and (b) thalamic ROI derived from the results of paired *t*-test analysis of therapeutic CPAP group. Scan1 = baseline, Scan2 = after treatment.

4.2. CVR and treatment effects in OSAS patients

No brain regions displayed significant group (sham vs. therapeutic CPAP) x scan (baseline vs. post-treatment) interaction in OSAS patients.

Paired within-group *t*-tests showed significantly increased CVR in the bilateral thalami after CPAP treatment and a significant decrease of CVR in the bilateral medial frontal/anterior cingulate cortex region after sham-CPAP treatment. No region showed a decrease in CVR after therapeutic CPAP treatment and no increase in CVR after sham-CPAP treatment (Table 2C, Fig. 1). Further exploratory analysis demonstrated that the anterior cingulate region that showed a significant decrease in CVR after sham CPAP treatment also showed a significant group versus scan interaction in OSAS patients at $p < 0.001$, uncorrected for multiple comparisons.

4.3. Multiple regressions for neural and clinical variables and CVR

Significant associations between clinical variables and CVR as reflected by CVR changes were observed in a number of brain areas. We found that AHI was positively correlated with CVR in the left posterior cingulate gyrus/precuneus (BA 30), extending to bilateral lingual gyri (BA 18). Conversely, the AHI was significantly negatively correlated with CVR in the right thalamus.

A second clinical parameter of interest in our patient group was the duration of nocturnal hypoxemia, as this is thought to be related to OSAS-related cellular injury. We observed a significant positive correlation between CVR and the duration of nocturnal hypoxemia in the bilateral paracentral lobules and precuneus (BA 5, 7). By contrast, we observed significant negative correlations between CVR and the duration of nocturnal hypoxemia in the left parahippocampal gyrus, the right middle (BA 8) and inferior frontal (BA 9) gyri, the right middle and inferior temporal (BA 21) gyri, extending to posterior insula and parahippocampal gyrus (Table 2B, Fig. 1).

Relevant to the possible relationship between OSAS and-BMI, there was no significant positive correlation between CVR and BMI. By comparison, significant negative correlation between CVR and BMI was found in the bilateral parahippocampal gyri and left cingulate gyrus (Table 2B).

4.4. Cerebral perfusion (rCBF) in OSAS patients versus healthy controls

We observed no significant difference ($p = 0.05$, corrected for multiple comparisons) in perfusion between OSAS patients and healthy controls (mean global rCBF values: 129.9 ± 22.1 vs. 113.5 ± 15, corresponding to perfusion values of 13 ± 2.2 and 11.4 ± 1.5 ml/100 g/min, respectively) and no treatment group versus session interaction (mean global rCBF values for therapeutic CPAP group: 127.9 ± 14.1 (baseline) and 115.6 ± 15 (after treatment), corresponding to perfusion values of 12.8 ± 1.4 and 11.6 ± 1.5 ml/100 g/min; and sham CPAP group: 132.5 ± 27.6 (baseline) and 114.1 ± 25.2 (after treatment), corresponding to perfusion values of 13.3 ± 2.8 and 11.4 ± 2.5 ml/100 g/min, respectively).

4.5. rCBF and BH-fMRI correspondence

To better understand the relationship between CVR and rCBF measures obtained in this sample, we assessed the relation between these two variables in the gray matter of brain areas included in FAIR slices (temporal lobes, basal ganglia inferior and middle regions of frontal cortex). We found no correlation between CVR and rCBF values in the above gray matter structures of the OSAS patients.

5. Discussion

To the best of our knowledge, this is the first study investigating CVR and cerebral perfusion as well as their changes in response to therapeutic and sham CPAP treatments in OSAS patients using whole-brain functional magnetic resonance imaging approach. There are four main findings in our study: (a) OSAS patients have significantly lower CVR as compared to healthy controls in several brain regions and vascular territories, (b) CVR is negatively correlated with the duration of nocturnal hypoxemia and BMI in several cortical regions, and particularly in bilateral hippocampi/parahippocampal gyri, (c) CVR increases with therapeutic CPAP but decreases with sham CPAP in OSAS patients, and (d) there was no correlation between CVR and rCBF and no difference in rCBF between patients and healthy controls.

Our findings are in agreement with a previous transcranial Doppler study [37], which found impaired CVR in OSAS patients as compared to healthy controls (HC), as well as an improvement in CVR after therapeutic CPAP treatment. However, the whole-brain approach extended those observations beyond the vascular territory of the middle cerebral artery and allowed specific spatial localization, which led to the important finding that CVR deficits are neither homogenous nor follow major vascular territories, but rather appear to be related to underlying neuronal networks.

In particular, several CVR changes that occurred after CPAP and sham CPAP treatment were found in regions that constitute the so-called "Default Mode Network" (DMN). DMN is described as a set of brain regions with correlated activity and which show deactivation of BOLD signal during externally oriented tasks [38–40].

The "deactivation" is related to re-allocation of cerebral perfusion to cortical areas engaged in the particular goal-oriented task.

In our previous work, we reported that OSAS preferentially affects the deactivation within DMN brain regions during a working memory task, in direct association with impaired behavioral performance, while activation within the fronto-parietal network was relatively preserved [41]. In the present study, OSAS patients also showed a decreased CVR in the left putamen/parahippocampal gyrus as compared to HC, a decreased CVR in the medial prefrontal cortex after sham-CPAP treatment (with a trend for significant group x scan interaction), as well as a negative correlation between CVR and duration of nocturnal hypoxemia in the temporal cortex. All these regions overlap with the DMN of our HC (as our HC group also participated in a working memory task protocol from which DMN brain mask was determined). The meaning of lower CVR values in OSAS patients in DMN regions is still unclear. Considering that DMN regions exhibit decreased signal during goal-oriented tasks, which is interpreted as reallocation of blood flow to those regions, our observation can indicate that in OSAS patients some degree of blood flow reallocation is already present during restful wakefulness.

Aside from DMN regions, we found an improvement (increase) in CVR after 2 months of therapeutic CPAP treatment in bilateral thalami. The thalamus is involved in maintaining both arousal and attention levels and its activation was found to increase during low-arousal attentional tasks and after sleep deprivation [42,43]. Therefore, decreased CVR in basal ganglia of OSAS patients before CPAP treatment likely reflects higher baseline activation of those regions, which is not revealed by relative BOLD signal changes during cognitive tasks, and which is decreased after therapeutic CPAP treatment.

Previous studies have proposed that nocturnal hypoxemia is the major culprit in CVR dysregulation observed in OSAS patients [7,45]. Hippocampus is known to be particularly vulnerable to hypoxic injury and is the structure that is most consistently affected in structural and metabolic studies of OSAS patients. In our study, the most prominent negative correlation between CVR changes and the duration of nocturnal hypoxemia was found in bilateral medial temporal regions (hippocampi/parahippocampal gyri). This observation is in accordance with our recently published finding that longer duration of nocturnal hypoxemia led to an impaired deactivation of temporal regions of the DMN during a working memory task [44]. Therefore, from these and prior data we hypothesize that CVR is influenced not only by intrinsic vascular factors, but also by the degree of neuronal activity of the surrounding gray. Based on our data, we propose that the observed CVR changes in OSAS patients do not only reflect endothelial/vascular dysfunction but rather a parallel underlying baseline neuronal function, which is affected by OSAS, particularly in the DMN brain regions. Integrating these findings in a progressive untreated OSAS evolution pattern, we suggest that baseline neuronal activation changes will lead to more permanent vascular changes increasing cerebrovascular risk by decreasing vascular reserve, despite the fact that we cannot completely rule out with our current results, the unlikely possibility that impaired CVR leads to changes in neuronal activity.

Our results also strongly suggest that compromised CVR is one of the mechanisms ultimately mediating hippocampal vulnerability to hypoxemia in OSAS. They call for further studies examining the causal relationship between nocturnal hypoxemia, CVR impairment, and structural and functional abnormalities of the hippocampus.

We also found negative correlation between BOLD signal changes and BMI in bilateral parahippocampal corci. Both nocturnal hypoxemia and overweight/obesity impact CVR of medial temporal structures, indicating that obesity and OSAS have a synergistic negative effect on CVR.

There has been a recent and increasing interest in the role of obesity as an independent risk factor for cognitive and cerebrovascular risk factor [46–49]: Increased BMI has been associated with reduced blood flow velocities and increased cerebral vascular resistance, in non-apneic subjects [50]. Recently we demonstrated a significant negative correlation between BMI and cerebral activation during a working memory task in OSAS patients, independently of AHI and nocturnal hypoxemia [41]. Therefore, treatment of obesity in conjunction with treatment of OSAS should have a synergistic positive effect on both cognitive function and CVR.

In this study, subjects were pre-selected to have no cardiovascular comorbidities in order to assess the roles of OSAS-related factors and obesity independently of cardiovascular comorbidity. Three previous Doppler studies of vascular reactivity in OSAS patients without cardiovascular comorbidities did not find differences between OSAS patients and HC [51–53]. Therefore, our findings of CVR differences between untreated OSAS patients and HC suggest that BH-fMRI may be more sensitive to CVR changes than transcranial Doppler.

Our study has several limitations. We acknowledge that previous transcranial Doppler studies had shown a maximal impact on CVR occurring in the morning after awakening, with a progressive recovery of CVR from morning to afternoon hours [8] and the fact that our observation was made with scanning in the evening, thus minimizing OSAS impact on CVR, and possibly diminishing our ability to detect important differences in therapeutic and sham CPAP treatment effects. Our choice of evening session was motivated by the desire to determine whether CVR differences were still detectable at the end of the day. Future investigations looking at the effect of circadian rhythm combining fMRI with other perfusion imaging techniques are justified.

The fact that we had no arterial gas measurements during the task allows for greater variability of CO_2 response during the task among subjects, potentially weakening our ability to detect significant results. However, BH-fMRI has been previously shown to have a good correlation with acetazolamide studies and therefore we feel that this approach is valid for measuring CVR. Nevertheless, future studies controlling for SaO_2 and $SaCO_2$ changes and using them for BOLD signal convolution could improve and expand our findings.

6. Conclusions

CVR changes in OSAS patients are detectable with BH-fMRI and are mostly present in basal ganglia and DMN regions. Observed CVR changes are neither homogenous throughout the brain nor follow vascular territories, but rather correspond to underlying neuronal networks, establishing a relationship between CVR and surrounding neuronal activity. The duration of nocturnal hypoxemia and the BMI negatively correlate with CVR, particularly in the medial temporal structures strongly supporting a pathophysiological mechanism for hippocampal injury in OSAS. Additionally, therapeutic and sham CPAP treatments have opposite effects on CVR of OSAS patients, with exacerbation of OSAS effects over time in the sham CPAP treatment group.

Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2014.04.004.

Acknowledgments

Olga Prilipko was supported by research grants from Swiss National Foundation for Scientific Research and FSBMB. This research is part of Dr. Olga Prilipko's Ph.D thesis (C. Guilleminault,

Director). This study was supported by an unrestricted research grant from Respironics, Resmed, and Covidien.

References

[1] Young T, Peppard PE, Gottlieb DJ. Epidemiology of obstructive sleep apnea: a population health perspective. Am J Respir Crit Care Med 2002;165:1217–39.
[2] Culebras A. Cerebrovascular disease and the pathophysiology of obstructive sleep apnea. Curr Neurol Neurosci Rep 2007;7:173–9.
[3] Nowak M, Kornhuber J, Meyrer R. Daytime impairment and neurodegeneration in OSAS. Sleep 2006;29:1521–30.
[4] Nasr N, Traon AP, Czosnyka M, Tiberge M, Schmidt E, Larrue V. Cerebral autoregulation in patients with obstructive sleep apnea syndrome during wakefulness. Eur J Neurol 2009;16:386–91.
[5] Reichmuth KJ, Dopp JM, Barczi SR, Skatrud JB, Wojdyla P, Hayes Jr D, et al. Impaired vascular regulation in patients with obstructive sleep apnea: effects of continuous positive airway pressure treatment. Am J Respir Crit Care Med 2009;180:1143–50.
[6] Furtner M, Staudacher M, Frauscher B, Brandauer E, Esnaola y Rojas MM, Gschliesser V, et al. Cerebral vasoreactivity decreases overnight in severe obstructive sleep apnea syndrome: a study of cerebral hemodynamics. Sleep Med 2009;10:875–81.
[7] Morgan BJ, Reichmuth KJ, Peppard PE, Finn L, Barczi SR, Young T, et al. Effects of sleep-disordered breathing on cerebrovascular regulation: a population-based study. Am J Respir Crit Care Med 2010;182:1445–52.
[8] Placidi F, Diomedi M, Cupini LM, Bernardi G, Silvestrini M. Impairment of daytime cerebrovascular reactivity in patients with obstructive sleep apnoea syndrome. J Sleep Res 1998;7:288–92.
[9] Diomedi M, Placidi F, Cupini LM, Bernardi G, Silvestrini M. Cerebral hemodynamic changes in sleep apnea syndrome and effect of continuous positive airway pressure treatment. Neurology 1998;51:1051–6.
[10] Kastrup A, Dichgans J, Niemeier M, Schabet M. Changes of cerebrovascular CO_2 reactivity during normal aging. Stroke 1998;29:1311–4.
[11] Kastrup A, Li TQ, Takahashi A, Glover GH, Moseley ME. Functional magnetic resonance imaging of regional cerebral blood oxygenation changes during breath holding. Stroke 1998;29:2641–5.
[12] Kastrup A, Kruger G, Glover GH, Neumann-Haefelin T, Moseley ME. Regional variability of cerebral blood oxygenation response to hypercapnia. Neuroimage 1999;10:675–81.
[13] Kastrup A, Li TQ, Glover GH, Moseley ME. Cerebral blood flow-related signal changes during breath-holding. AJNR Am J Neuroradiol 1999;20:1233–8.
[14] Rostrup E, Law I, Blinkenberg M, Larsson HB, Born AP, Holm S, et al. Regional differences in the CBF and BOLD responses to hypercapnia: a combined PET and fMRI study. Neuroimage 2000;11:87–97.
[15] Riecker A, Grodd W, Klose U, Schulz JB, Groschel K, Erb M, et al. Relation between regional functional MRI activation and vascular reactivity to carbon dioxide during normal aging. J Cereb Blood Flow Metab 2003;23:565–73.
[16] Thomason ME, Burrows BE, Gabrieli JD, Glover GH. Breath holding reveals differences in fMRI BOLD signal in children and adults. Neuroimage 2005;25:824–37.
[17] Shiino A, Morita Y, Tsuji A, Maeda K, Ito R, Furukawa A, et al. Estimation of cerebral perfusion reserve by blood oxygenation level-dependent imaging: comparison with single-photon emission computed tomography. J Cereb Blood Flow Metab 2003;23:121–35.
[18] Kamba M, Suto Y, Ohta Y, Inoue Y, Matsuda E. Cerebral metabolism in sleep apnea. Evaluation by magnetic resonance spectroscopy. Am J Respir Crit Care Med 1997;156:296–8.
[19] Alchanatis M, Deligiorgis N, Zias N, Amfilochiou A, Gotsis E, Karakatsani A, et al. Frontal brain lobe impairment in obstructive sleep apnoea: a proton MR spectroscopy study. Eur Respir J 2004;24:980–6.
[20] Bartlett DJ, Rae C, Thompson CH, Byth K, Joffe DA, Enright T, et al. Hippocampal area metabolites relate to severity and cognitive function in obstructive sleep apnea. Sleep Med 2004;5:593–6.
[21] Morrell MJ, McRobbie DW, Quest RA, Cummin AR, Ghiassi R, Corfield DR. Changes in brain morphology associated with obstructive sleep apnea. Sleep Med 2003;4:451–4.
[22] Torelli F, Moscufo N, Garreffa G, Placidi F, Romigi A, Zannino S, et al. Cognitive profile and brain morphological changes in obstructive sleep apnea. Neuroimage 2011;54:787–93.
[23] O'Donoghue FJ, Wellard RM, Rochford PD, Dawson A, Barnes M, Ruehland WR, et al. Magnetic resonance spectroscopy and neurocognitive dysfunction in obstructive sleep apnea before and after CPAP treatment. Sleep 2012;35:41–8.
[24] Kim SG. Quantification of relative cerebral blood flow change by flow-sensitive alternating inversion recovery (FAIR) technique: application to functional mapping. Magn Reson Med 1995;34:293–301.
[25] Kim SG, Tsekos NV. Perfusion imaging by a flow-sensitive alternating inversion recovery (FAIR) technique: application to functional brain imaging. Magn Reson Med 1997;37:425–35.
[26] Farre R, Hernandez L, Montserrat JM, Rotger M, Ballester E, Navajas D. Sham continuous positive airway pressure for placebo-controlled studies in sleep apnoea. Lancet 1999;353:1154.
[27] Thomason ME, Glover GH. Controlled inspiration depth reduces variance in breath-holding-induced BOLD signal. Neuroimage 2008;39:206–14.
[28] Glover GH, Law CS. Spiral-in/out BOLD fMRI for increased SNR and reduced susceptibility artifacts. Magn Reson Med 2001;46:515–22.
[29] Glover GH, Lai S. Self-navigated spiral fMRI: interleaved versus single-shot. Magn Reson Med 1998;39:361–8.
[30] Kim DH, Adalsteinsson E, Glover GH, Spielman DM. Regularized higher-order in vivo shimming. Magn Reson Med 2002;48:715–22.
[31] Preston AR, Thomason ME, Ochsner KN, Cooper JC, Glover GH. Comparison of spiral-in/out and spiral-out BOLD fMRI at 1.5 and 3 T. Neuroimage 2004;21:291–301.
[32] Thomason ME, Waugh CE, Glover GH, Gotlib IH. COMT genotype and resting brain perfusion in children. Neuroimage 2009;48:217–22.
[33] Macey PM, Macey KE, Kumar R, Harper RM. A method for removal of global effects from fMRI time series. Neuroimage 2004;22:360–6.
[34] Song XW, Dong ZY, Long XY, Li SF, Zuo XN, Zhu CZ, et al. REST: a toolkit for resting-state functional magnetic resonance imaging data processing. PLoS One 2011;6:e25031.
[35] Ledberg A, Akerman S, Roland PE. Estimation of the probabilities of 3D clusters in functional brain images. Neuroimage 1998;8:113–28.
[36] Glascher J. Visualization of group inference data in functional neuroimaging. Neuroinformatics 2009;7:73–82.
[37] Jimenez Caballero PE, Coloma Navarro R, Ayo Martin O, Segura Martin T. Cerebral hemodynamic changes in obstructive sleep apnea syndrome after continuous positive airway pressure treatment. Sleep Breath 2013;17:1103–8.
[38] Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. Proc Natl Acad Sci U S A 2001;98:676–82.
[39] Gusnard DA, Raichle ME. Searching for a baseline: functional imaging and the resting human brain. Nat Rev Neurosci 2001;2:685–94.
[40] Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. Ann N Y Acad Sci 2008;1124:1–38.
[41] Prilipko O, Huynh N, Schwartz S, Tantrakul V, Kim JH, Peralta AR, et al. Task positive and default mode networks during a parametric working memory task in obstructive sleep apnea patients and healthy controls. Sleep 2011;34:293A–301A.
[42] Portas CM, Rees G, Howseman AM, Josephs O, Turner R, Frith CD. A specific role for the thalamus in mediating the interaction of attention and arousal in humans. J Neurosci 1998;18:8979–89.
[43] Tomasi D, Wang RL, Telang F, Boronikolas V, Jayne MC, Wang GJ, et al. Impairment of attentional networks after 1 night of sleep deprivation. Cereb Cortex 2009;19:233–40.
[44] Prilipko O, Huynh N, Schwartz S, Tantrakul V, Kushida C, Paiva T, et al. The effects of CPAP treatment on task positive and default mode networks in obstructive sleep apnea patients: an fMRI study. PLoS One 2012;7:e47433.
[45] Duchna HW, Guilleminault C, Stoohs RA, Faul JL, Moreno H, Hoffman BB, et al. Vascular reactivity in obstructive sleep apnea syndrome. Am J Respir Crit Care Med 2000;161:187–91.
[46] Wolf PA, Beiser A, Elias MF, Au R, Vasan RS, Seshadri S. Relation of obesity to cognitive function: importance of central obesity and synergistic influence of concomitant hypertension. The Framingham Heart Study. Curr Alzheimer Res 2007;4:111–6.
[47] Cournot M, Marquie JC, Ansiau D, Martinaud C, Fonds H, Ferrieres J, et al. Relation between body mass index and cognitive function in healthy middle-aged men and women. Neurology 2006;67:1208–14.
[48] Gazdzinski S, Millin R, Kaiser LG, Durazzo TC, Mueller SG, Weiner MW, et al. BMI and neuronal integrity in healthy, cognitively normal elderly: a proton magnetic resonance spectroscopy study. Obesity (Silver Spring) 2010;18:743–8.
[49] Gunstad J, Paul RH, Cohen RA, Tate DF, Spitznagel MB, Gordon E. Elevated body mass index is associated with executive dysfunction in otherwise healthy adults. Compr Psychiatry 2007;48:57–61.
[50] Selim M, Jones R, Novak P, Zhao P, Novak V. The effects of body mass index on cerebral blood flow velocity. Clin Auton Res 2008;18:331–8.
[51] Tonini M, Khayi H, Pepin JL, Renard E, Baguet JP, Levy P, et al. Choroidal blood-flow responses to hyperoxia and hypercapnia in men with obstructive sleep apnea. Sleep 2010;33:811–8.
[52] Urbano F, Roux F, Schindler J, Mohsenin V. Impaired cerebral autoregulation in obstructive sleep apnea. J Appl Physiol 2008;105:1852–7.
[53] Foster GE, Hanly PJ, Ostrowski M, Poulin MJ. Ventilatory and cerebrovascular responses to hypercapnia in patients with obstructive sleep apnoea: effect of CPAP therapy. Respir Physiol Neurobiol 2009;165:73–81.